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(54) BLOOD-POOL IMAGING COMPOSITIONS USE AND METHOD

BLUT-POOL BILDERZEUGUNGS-ZUSAMMENSETZUNGEN DEREN HERSTELLUNG UND VERWENDUNG

COMPOSITIONS D'IMAGERIE DE POOL SANGUIN ET PROCEDE

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(73) Proprietor: **BRACCO RESEARCH S.A.**
1228 Plan-les-Ouates (CH)

(72) Inventors:
- **TOURNIER, Hervé**
  F-74520 Valleiry (FR)
- **LAMY, Bernard**
  CH-1203 Geneva (CH)
- **HYACINTHE, Roland**
  F-74140 Douvaine (FR)

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- **CHEMICAL ABSTRACTS, vol. 110, no. 17, 24 April 1989 Columbus, Ohio, US; abstract no. 150611, SCHWENDENER, R. A. ET AL: "Small unilamellar liposomes as magnetic resonance contrast agents loaded with paramagnetic manganese-, gadolinium-, and iron-DTPA-stearate complexes" XP002014218 cited in the application & INT. J. PHARM. (1989), 49(3), 249-59 CODEN: IJPHDE;ISSN: 0378-5173,**
- **MAGNETIC RESONANCE IN MEDICINE, vol. 27, no. 1, pages 44-51, XP000297603 TILCOCK C ET AL: "THE DESIGN OF LIPOSOMAL PARAMAGNETIC MR AGENTS: EFFECT OF VESICLE SIZE UPON THE RELAXIVITY OF SURFACE-INCORPORATED LIPOPHILIC CHELATES"**

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Description

[0001] The invention relates to NMR imaging contrast compositions comprising as components of a dispersion in a physiologically acceptable aqueous carrier phase a paramagnetic metal ion coupled to a chelating agent having a lipophilic moiety, a physiologically acceptable non-ionic surfactant or a mixture of non-ionic surfactants and optionally one or more amphipatic organic compounds, the components of the dispersion being in a micellar form. The invention also concerns the preparation of the compositions, as well as injectable MRI blood pool contrast agents, their use and a kit comprising dry contrast composition and a physiologically acceptable aqueous carrier.

Background art

[0002] In general, the relatively low molecular weight magnetically responsive water-soluble metal complexes such as Gd-DTPA, Gd-DOTA etc. are not suitable for use as contrast agents for blood-pool imaging due to their partial leakage through the vessel walls (extravasation into the extravascular space) and their very rapid elimination through the kidneys. The rapid elimination renders these substances unsuitable for imaging of the vascular system since they cannot provide acceptable contrasts (decrease of T_1 relaxation time of protons) for a sufficient time.

[0003] Various attempts to produce substances suitable as MRI contrast agents for blood-pool investigations have been made. Search for contrast agents with long residence times in the blood circulation, high relaxivity and complete elimination of substances administered have brought proposals in which paramagnetic substances are encapsulated into liposome vesicles or immobilised in the liposome membrane, copolymerised with polyethylene glycol or grafted on a polymeric chain such as albumin, dextran or polylysine. Examples of such compositions are Gd-DTPA-albumin, Gd-DTPA-dextran or Gd-DTPA-polylysine complex molecules (see for example: M.D. Ogan et al., Invest. Radiol. **22** (1987) 665; S.C. Wang et al., Radiology **175** (1990) 483; G. Schumann-Giampieri et al., Invest. Radiol. **26** (1991) 969; and A.V.S. Vexler et al. Invest. Radiol. **29** supl. 2 (1994) S62; B. T.S. Dessler et al., Invest. Radiol. **29** supl. 2 (1994) S65; C. D. Meyer et al., Invest. Radiol. **29** supl. 2 (1994) S90; D. D. Shen et al., Invest. Radiol. **29** supl. 2 (1994) S217). The aforementioned compositions exhibit longer dwelling times in the blood than the water-soluble metal complexes, however, their residence times in the circulation are still not sufficient and some of these compounds have shown unacceptable levels of toxicity for blood-pool imaging. Longer residence times and lower immunogenicity have been reported by A.A. Bogdanov et al., Radiology **187** (1993) 701 for Gd-DTPA-MPEG-polylysine complexes which consist of a methoxy poly(ethylene glycol)-shielded macromolecular backbone (polylysine) bearing covalently attached Gd-DTPA.

[0004] Among the many approaches for enhancing the relaxivities of paramagnetic substances in the blood, of interest may be the proposal made in WO-A-91/14178 (Research Corporation). This document discloses paramagnetic contrast enhancing agents which are lipophilic in nature and are based on polyaminopolycarboxylic acid derivatives especially EDTA and DTPA derivatives having one or two fatty acid moieties and a carboxymethylacetamide replacing at least one acetic acid group and preferably two acetic acid groups. The preferred paramagnetic metal ions are the usual paramagnetic metal ions including gadolinium. Conjugates of the paramagnetic contrast agents with other physiological agents such as proteins, peptides, antibodies or liposomes have also been disclosed. The lipophilic paramagnetic agents can be incorporated into liposome membrane to assist targeting and improve the relaxivity.

[0005] Notwithstanding, the half-life of contrast agents containing paramagnetic species bonded to macromolecules is often too short to be convenient for blood-pool imaging. In order to solve this difficulty, the use of suspensions of liposomal microvesicles containing encapsulated paramagnetic chelates as carriers of NMR contrast agents has been proposed. Use of liposomes for carriers has been proposed for relative biocompatibility and ease of preparation of liposomes and their suspensions. Encapsulation of known paramagnetic contrast agents into liposomes has been described in a number of different publications (e.g. E.C. Unger et al. JMRI 3 (1993), 195-198, EP-A-0 160 552, etc.).

[0006] Unfortunately, the useful life of liposome encapsulated contrast agents injected in the circulation is short because of the rapid physiological removal due to opsonization followed by phagocytosis. The opsonization process involves the coating of "intruder" particles by proteins, called opsonins, recognisable by macrophages followed by their removal (phagocytosis) and metabolization of the coated (opsonized) particles by the Kupffer cells of the liver and the spleen.

[0007] Hence, liposomes as carriers of water-soluble paramagnetic chelates do not constitute an ideal solution to paramagnetic blood pool contrast agents. As said before most liposomes are subject to rapid removal from the circulation by the liver and the spleen and, although this property may be advantageous for imaging the latter organs, it is strongly undesirable when one wishes to keep the concentrations of contrast compounds in the blood at a relatively high level for a more extended time. Remedies have been proposed to prolong the life of liposomes vesicles in the blood, namely to incorporate protective substances in the vesicle forming lipids. Along this line of approach, "stealth factors", for instance covalently modified lipids, i.e. lipids (phosphatidylethanol amine (PE)) carrying grafted thereon externally extending polyethylene glycol (PEG) segments have been proposed. Also, the incorporation, as "stealth" factors,

to the vesicle forming lipids of products such as palmitoylglucuronic acid (PGlcUA) has been reported to improve the half-life of liposomes in the blood.

[0008] It is well known that the lifetime of liposomes in the blood may be significantly prolonged by making the liposome vesicles very small e.g. 50 nm or less. The suggestion is based on the fact that small particles are less size-recognisable by opsonins; therefore if the vesicles are sufficiently small, their residence time in the blood will increase. The trouble with very small vesicles, however, is that with reduction in size their entrapment capacity becomes very small which is not compatible with the amounts of contrast media required for imaging the blood-pool with paramagnetic compounds. Another drawback of liposomes is that the presence of the lipid membrane markedly shields the action of the contrast agent on the water protons within the investigation site. Although this negative effect can be reduced by incorporating the contrast agent within the membrane lipids, for instance by grafting a lipophilic group to the chelatant of the contrast agent (see R.A. Schwendener et al. Internat. J. Pharm. **49** (1989), 249-59), the results have been still insufficient up to now, the ratio of magnetically active substance to substrate being still relatively low and the residence time in the blood relatively short.

[0009] Hence the residence time of known paramagnetic MRI contrast agent compositions is still insufficient which renders these agents relatively ineffective when organ perfusion and blood volume measurements/imaging are required. Furthermore, although the longitudinal relaxivity r_1 or $(1/T_1)$ of the known agents is acceptable, further increase of this factor could provide even better contrast and resolutions, hence better imaging and/or would provide more effective agents requiring administration of lower amounts of imaging substances for the same quality and image resolution. Lowering the amount of the contrast agent administered would lead to even lower level of toxicity. Thus, providing a paramagnetic blood-pool contrast composition/agent which has a substantive action on the relaxation time T_1 of water protons, sufficient stealth properties for blood-pooling i.e. a life-time sufficient for effecting complete imaging with only one dose of injected composition, together with a very low or no immunogenicity and an optimal mole ratio of MRI responsive substance to pharmaceutically acceptable organic substrate is still very desirable in order to minimise possible after-injection side-effects.

Summary of the Invention

[0010] In brief, the invention relates to the paramagnetic, MRI responsive contrast compositions comprising as components of a dispersion in a suitable aqueous carrier liquid, a paramagnetic metal ion, a chelating agent having a lipophilic moiety, and a physiologically acceptable non-ionic surfactant or a mixture of non-ionic surfactants, wherein the non-ionic surfactant is a block-copolymer having polyoxyethylene and polyoxypropylene segments, a polyethyleneglycolalkylether, a polyoxyethylene fatty acid ester, an n-alkylglucopyranoside, or an n-alkyl maltotrioside; the components of the dispersion being in a micellar form. Optionally, the composition may include one or more amphipatic compounds e.g. phospholipids. The chelating agent comprises a polyamino-polycarboxylate backbone carrying at least one lipophilic substituent e.g. an ester of a fatty alcohol. Complexes of paramagnetic metal ions with the chelating agents are referred to as the imaging agents. The compositions of the invention are associations of imaging agents, non-ionic surfactants, and optionally phospholipids, into stable mixed micelles suspended in a suitable carrier liquid. The mixed micelles are constituted by conjugation or association of the imaging agent with non-ionic surfactant and optionally an amphipatic compound. The term association or conjugation means that the components of the micelles may be in the form of adducts or admixtures of two or more substances having mutual affinity; or the association may be due to one or more bonds e.g. H-bonds between the constituents, whereby a chelatant molecule with simultaneous lipophilic and hydrophilic properties will be provided in a given desirable equilibrium (appropriate hydrophilic/lipophilic balance). Hence, the imaging composition may comprise a mixture of a substrate having suitable amphiphilic properties, and a compound including a paramagnetic species and a function possessing affinity for the substrate; or the imaging composition may comprise a more or less loose adduct of the foregoing constituents.

[0011] Clearly, the presence of the non-ionic surfactant or mixtures of non-ionic surfactants in the composition is essential since the non-ionic surfactant causes the principal constituents i.e. the paramagnetic metal ion and the chelating agent having a lipophilic function, the phospholipid and the surfactant to form mixed micelles. By rendering the principal constituents of the composition micellar the properties of the constituents change and unexpectedly effective imaging properties are obtained. The size of the micelles is found to vary between 10 and 800 nm, however, it appears that the most effective results are obtained when the size is preferably between 30 and 500 nm. Dispersed in a suitable aqueous carrier liquid, the mixed micelles form very stable colloidal dispersions which resist agglomeration or aggregation for a long period.

[0012] The invention also relates to a method of making the paramagnetic contrast compositions comprising non-ionic surfactants, their use as blood pool contrast agents, and a method of manufacture of contrast agents as dry powders obtained by lyophilisation of the composition.

[0013] A kit comprising a vial with dry pulverulent formulation obtained by lyophilisation of the composition and optionally a vial with an aqueous physiologically acceptable carrier is also disclosed.

Brief description of the drawings

[0014]

Fig. 1 is a schematic presentation of a mixed micelle of the composition comprising a paramagnetic metal ion 1, a chelating agent having a lipophilic moiety 2, a phospholipid 3 and a physiologically acceptable non-ionic surfactant 4.

Fig. 2 is a graph showing comparative data of T_1 - Relaxivity in water obtained for Gd-DTPA, various Gd-based macromolecular agents and the micellar Gd-DTPA-SE/DPPA/F108, Gd-DTPA-$(SE)_2$/DPPA/BRIJ® 78 and Gd-DTPA-$(SE)_2$/BRIJ®78 compositions according to the invention.

Fig. 3 is a schematic presentation of structural formula of the amphipatic derivative of DTPA [DTPA-$(SE)_2$] prepared via reaction of DTPA anhydride with stearyl alcohol.

Fig. 4 is a diagram of blood pharmacokinetics in the rat of a micellar Gd-DTPA-SE/phospholipid/F108 composition according to the invention.

Fig. 5 is a diagram of blood pharmacokinetics in the rat of a micellar Gd-DTPA-$(SE)_2$/phospholipid/F108 composition according to the invention

Fig. 6 is a diagram of blood pharmacokinetics in the rat of micellar radio labelled compositions with different phospholipids produced according to the invention.

Fig. 7 is a diagram of blood pharmacokinetics in the rat of micellar ^{153}Gd-DTPA-$(SE)_2$/DPPC/BRIJ® 78 and ^{153}Gd-DTPA-$(SE)_2$/BRIJ® 78 compositions according to the invention.

Detailed description of the Invention.

[0015] The main aspects of the invention as set out in the accompanying claims are based on an unexpected finding that exceptionally effective paramagnetic NMR contrast compositions are obtained when in addition to a paramagnetic metal ion complexed with a polyaminopolycarboxylate chelating agent having a lipophilic moiety, the imaging composition comprises a physiologically acceptable non-ionic surfactant or a mixture of non-ionic surfactants and preferably one or more amphipatic compounds such as phospholipids. The paramagnetic metal ion is complexed with the polyaminopolycarboxylate and the complex is often referred to as imaging agent. This notwithstanding that only paramagnetic ion has the desired magnetic properties and is therefore almost solely responsible for the imaging action i.e. change in relaxivity of the hydrogen atoms of water. The complexing of the metal ion and hence the presence of the chelating agent is required only to counteract the toxicity of the paramagnetic metal ions and eliminate their undesired effects. Amongst chelating agents derivatives polyaminopolycarboxylic acids are found to be particularly useful for complexing the paramagnetic ions intended for NMR imaging of human or animal body.

[0016] In the compositions according to the invention, the polyaminopolycarboxylate chelating agent is provided with a hydrophobic group (for instance, an esterified fatty alcohol chain) which readily couples or intertwines (presumably by Van der Waals forces) with the hydrophobic part of non-ionic surfactant and optionally with the fatty acid residues of the phospholipid. The non-ionic surfactant presumably provides the additional hydrophilic/lipophilic balance parameters to enable the four component system to exist as mixed micelles dispersed in a carrier liquid.

[0017] As schematically presented in Fig. 1, said mixed micelles comprise a paramagnetic metal ion (1) retained by a chelating agent having a lipophilic moiety (2), an amphipatic compound e.g. a phospholipid (3) and a non-ionic surfactant (4). This configuration of a paramagnetic metal ion bonded to an amphipatic structure i.e. a polyaminopolycarboxylate segment comprising ionic hydrophilic functions, a non-ionic hydrophilic function (the polyethylene oxide segment) and non-ionic hydrophobic aliphatic chains has shown strikingly high contrast efficiency in NMR blood pool imaging. As it may be seen from the experimental part, this contrast effect is at least 30% better than that of comparative compositions of the prior art in which the phospholipid is laminar (vesicular form) instead of micellar. The exact reason why this configuration difference is so effective is still unexplained; however, it has been established that the mixed micelles may have particle sizes between 10 and 800 nm, best results being obtained with the micelles of size in the range between 30 and 500 nm.

[0018] A possible explanation of the exceptional properties of the mixed micelles of the invention and their suitability as MRI blood-pool contrast agents may come from the fact that they have simultaneous affinity for water and for oils, i.e. they possess suitable lipophilic/hydrophilic balance. The hydrophilic functions involved are ionic and non-ionic. The corresponding hydrophilic/lipophilic balance (HLB) may vary considerably and may be between 1 to 50, but is preferably from 5 to 15. It is speculated that due to these equilibrated surfactant properties, when the mixed micelles are dispersed in a suitable aqueous carrier liquid, they form very stable colloidal dispersions, i.e. the micelles resist agglomeration or aggregation into larger aggregates for a long period. The diagram presented in Fig. 2 shows relaxivity values T_1 obtained for the contrast compounds according to the invention and relaxivity values reported for Gd-DTPA and various Gd-based macromolecular agents. As it can be seen from this comparative diagram, the contrast

agents comprising paramagnetic contrast composition in the form of mixed micelles provides relaxivities, which are 30-250% greater than that of the heretofore known compositions. Thus, the higher relaxivities coupled to the longer residence times in the circulation obtained with the paramagnetic contrast agents of the invention provide an important advance (advantage) in comparison to the known NMR contrast agent compositions.

[0019] The mixed micelles according to the invention may be produced using non-ionic, ionic and mixtures of ionic and non-ionic surfactants however, due to their physiological suitability the non-ionic surfactants are preferred. The non-ionic surfactants are block-copolymers having polyoxyethylene and polyoxypropylene segments, polyethylene-glycolalkylethers such as for example polyethyleneglycol-octadecylether, or polyoxyethylene fatty acid esters, or n-alkyl glycopyranoside and n-alkyl maltotrioside. The non-ionic surfactant in the compositions of the invention is conveniently selected from the commercially available products such as Pluronic®, Poloxamer®, Poloxamine®, Synperonic®, BRIJ®, Myrj® and their mixtures. The weight proportion of the surfactant relative to the amount of the paramagnetic imaging agent is from 1:50 to 50:1, preferably 1:10 to 10:1, and even more preferably 1:1.

[0020] In order to make the imaging polycarboxylic chelating molecule compatible with the phospholipids and the non-ionic surfactants, the chelating molecule is provided with a hydrophobic group, for instance in the form of carboxylate ester with hydrophobic aliphatic or aromatic alcohols. As said alcohols, one may cite saturated and unsaturated C_1 to C_{24} alcohols like methanol, ethanol, propanol, butanol (n-, iso-, tert-), pentanol, hexanol (and isomers), heptanol, octanol (and isomers), nonanol, decanol and fatty alcohols; as aromatic alcohols, one may cite substituted and unsubstituted benzyl- and higher phenylalkyl-alcohols. The chelating molecule may also be provided with the hydrophobic group in form of a carboxylate amide with hydrophobic aliphatic or aromatic amines. Said amines may be saturated and unsaturated C_1 to C_{24} amines like methylamine, ethylamine, propylamine, butylamine (n-, iso-, tert-), pentylamine, hexylamine (and isomers), octylamine (and isomers), nonylamine, decylamine, aminoadamantan and fatty amines; as aromatic amines, one may cite substituted and unsubstituted benzyl- and higher phenylalkyl-amines. Alternatively, the polycarboxylic chelating agent can be provided with lipophilic hydrophobic groups linked to the alkylene segments of the molecular back-bone, or to the α-carbon of the carboxylate functions or to a hydroxyl group when present in the chelating agent. An example of the latter is the product of reaction between Gd-HP-DO3A with a fatty acid chloride.

[0021] Experiments have shown that the lipophilic moiety of the polyaminopolycarboxylate chelating agent may vary in the range from a methyl (C_1) to a long chain alkyl or alkylene group with as many as 24 carbon atoms (C_{24}) and may also include substituted or unsubstituted benzyl- or higher phenyl alkyl groups. In fact as long as the polycarboxylic chelate has a lipophilic function which presumably provides an anchor for the phospholipid and/or the surfactant molecules the mixed micelles are formed. The mixed micelles obtained seem reasonably stable even with short alkyl groups however for merely practical reasons alkyl groups with C_{12}-C_{18} are preferred. It has been found that when the non-ionic surfactant is eicosaethyleneglycol-octadecylether known under its trademark of BRIJ® 78 the presence of the phospholipid although beneficial in view of higher relaxivity is not really necessary, as the micelles of the surfactant and the paramagnetic complex are showing acceptable relaxivity and reasonable stability in the circulation.

[0022] The amphipatic compounds suitable in the present composition are phospholipids which may be selected from phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), cardiolipin (CL) and sphingomyelin (SM). The amphipatic compound may also consists of a mono-phosphate ester of a substituted or partially substituted glycerol, at least one functional group of said glycerol being esterified by saturated or unsaturated aliphatic fatty acid, or etherified by saturated or unsaturated alcohol, the other two acidic functions of the phosphoric acid being either free or salified with alkali or earth-alkali metals. Preferably the phosphate esters will include monophosphates of fatty acid glycerides selected from dimyristoylphosphatidic acid, dipalmitoylphosphatidic acid, or distearoylphosphatidic acid.

[0023] The phospholipids may also include diacyl and dialkyl glycerophospholipids in which the aliphatic chains have at least twelve carbon atoms, as well as one or more compounds selected from ionic and neutral phospholipids, mono alkyl or alkenyl esters of phosphoric acid and/or cholesterol, ergosterol, phytosterol, sitosterol, lanosterol, and tocopherol. In the compositions containing phospholipids, the weight proportion of the phospholipids to the polycarboxylic add chelate seems not critical and it may vary in a wide range e.g. from 1:50 to 50:1. The practical range will be between 10:1 and 1:10, preferably between 1:5 and 5:1 and even more preferably between 1:3 and 3:1 this since the use of a large excess of chelate may result in unnecessary waste of the chelating/imaging agent while an excess of phospholipid beyond certain concentration does not provide extra benefit. In the compositions in which phospholipids are used the weight ratio of the phospholipid to the surfactant may vary as above, however the ranges from 1:10 to 10:1 and preferably between 1:2 and 2:1 are considered as the ranges in which optimal compositions of the NMR blood pool agent are to be found.

[0024] The chelate moiety of the magnetically responsive component of the present micelles may be selected from EDTA, DTPA, BOPTA, DOTA, DO3A and/or their

derivatives, and the paramagnetic metal may be selected amongst well known group of paramagnetic metals notably from Gd(III), Mn(II), Cr(III), Cu(II), Fe (III), Pr(III), Nd(III) Sm(III), Tb(III), Yt(III) Dy(III), Ho(III) and Er(III).

[0025] It has also been established that a very useful form of the composition according to the invention may be produced by lyophilisation of the composition whereby a dry, pulverulent formulation is obtained. This form of the paramagnetic composition is particularly convenient for long term storage. The storage in the powder form is simplified by the fact that reconstitution of the composition comprising mixed micelles is achieved by dispersion of the lyophilised powder in a physiologically acceptable liquid carrier, will form a suspension useful as a blood pool NMR imaging contrast agent. The lyophilisation is a straight forward freeze-drying process requiring no particular precautions or measures.

[0026] The method for making compositions according to the invention comprises selecting as components a paramagnetic contrast agent with an appropriate polycarboxylic acid chelating agent provided with a suitable lipophilic group in admixture with one or more phospholipids and non-ionic surfactants and dispersing the components into micellar form in a physiologically acceptable aqueous liquid carrier such as water or saline, neat or buffered, according to usual practice. Depending upon the choice of components, the dispersion can be achieved by gentle mixing or by more energetic means such as homogenisation, microfluidization or sonication.

[0027] In an advantageous mode of performing the above preparation using for instance, as the required components, the mono- or di-stearyl ester of gadolinium DTPA, dipalmitoylphosphatidic acid (DPPA) as the phospholipid, and Synperonic® F-108 as a non-ionic surfactant, one part by weight of the contrast component is admixed with two parts each of the lipid and the surfactant, and 100 to 200 parts of water. The mixture is homogenised by sonication at a temperature of 50-80°C for a few minutes, until the dispersed mixtures forms mixed micelles mostly in the range of 20-250 nm. Generally, the micelles sizes distribution is Gaussian.

[0028] Alternatively, two components of the present particulate adduct, for instance the paramagnetic imaging component and the phospholipids, can be first dispersed in the aqueous carrier liquid and the third component added afterwards to the dispersion, whereby the addition of said third component will cause the dispersion to become into micellar form.

[0029] Hence, in an advantageous mode of carrying out this alternative, one part by weight of the paramagnetic component and two parts of the phospholipid are dissolved in a suitable organic solvent such as chloroform, methylene chloride, methanol, or mixtures thereof and the solution is evaporated to dryness under reduced pressure. Then the residual solid is finely dispersed in about 100 to 200 part of water (or other physiologically acceptable liquid carrier), for instance by sonication, microfluidization, or otherwise, about two parts of the F-108 surfactant (or of an equivalent compound) are added and homogenisation is resumed until micelles are formed as disclosed.

[0030] Once prepared, the dispersion can thereafter be sterilised by heat as usual and used as such, or it can be further dehydrated for storage, for instance by lyophilization. The dehydrated material in form of a powder from which the MRI contrast agent may be produced by admixing the powder with a portion of carrier liquid and shaking.

[0031] Thus, for practically applying the compositions of the invention in the medical field, the dried components and the carrier liquid can be marketed separately in a kit form whereby the contrast agent is reconstituted by mixing together the kit components prior to injection into the circulation of patients.

[0032] The first component of the kit i.e. dry power may further be stored under a dry inert atmosphere and the second component, a physiologically acceptable carrier liquid, may further contain isotonic additives and other physiologically acceptable ingredients such as various mineral salts, vitamins, etc.

[0033] As already mentioned the reconstituted agent is particularly suitable for use in NMR blood pool imaging of organs in human or animal body. These compositions could facilitate MR angiography and help to assess myocardial and cerebral ischemia, pulmonary embolism, vascularisation of tumours and tumour perfusion.

[0034] The following Examples further illustrate the invention.

Example 1

[0035] The DTPA mono- and di-stearyl esters of formulae, shown in Fig. 3, and the corresponding gadolinium chelates (Gd-DTPA-SE) and (Gd-DTPA-$(SE)_2$), were prepared as disclosed in G.W. Kabalka et al., Magnetic Resonance in Medicine 8 (1988), 89-95. The DTPA anhydride required in the synthesis was made according to Eckelman et al., J. Pharm. Sci. **64** (1975), 704-706. The purity of the imaging agents was checked by measuring the gadolinium content by usual means (decomplexing in 2N hydrochloric acid and titrating with EDTA solution; indicator, Xylenol-orange) and gave results substantially near theory.

[0036] Six hundred mg of lecithin (SPC-3, Natterman) (0.788 mmol), 60 mg cholesterol (0.158 mmol), and 332 mg Gd-DTPA-$(SE)_2$ (0.315 mmol) were dissolved in 100 ml of a 1/1 mixture of MeOH and $CHCl_3$. The solution was evaporated to dryness under reduced pressure (Rotavapor, 72°C/15 Torr, 1.5 hrs), after which 20 ml of distilled water were added under agitation. The mixture was further homogenised by sonication for about 30 min at 70°C (Branson Sonifier, output 40), whereby a homogenous milky suspension of liposome vesicles (labelled "L") was obtained.

[0037] To 10 ml of the above suspension were added 300 mg of Synperonic® F-108 and sonication was resumed for a few minutes, whereby a stable optically clearer suspension of submicronic particles (labelled "M") in micellar form was obtained.

TABLE 1

	r_1	r_2
"L"	10.6	8.6
"M"	20.6	13.2

[0038] Proton spin relaxivities of the foregoing suspensions were measured using a Minispec PC-120 (Bruker) apparatus, operating under 0.47 Tesla (20 MHz). EDM 510A (EDM = Experiment Definition Module) was used to measure the spin-lattice relaxation time T_1 by the "inversion recovery" method. EDM 610A was used to measure the spin-spin relaxation time T_2 by the Carr-Purcell-Meiboom-Gill (GPMG) technique. The relaxivities (r_1 and r_2) given in the Table 1 are expressed as r in $[mMs]^{-1}$ = 1/T for a 1 mM concentration.

[0039] The foregoing results clearly demonstrate that converting the imaging compound from vesicular to micellar from sharply increases relaxivity and consequently imaging efficiency.

Example 2

[0040] In a first preparative mode (mode 1), two samples were prepared by admixing together 100 mg of imaging agent, 200 mg of DPPA (dipalmitoylphosphatidic acid Na salt) and 200 mg of Synperonic® F-108 and 20 ml of H_2O, then the mixture was sonicated for 30 min at 70°C, (Branson sonifier output 40). In a first sample ("M1") there was used as the imaging species the monostearylester Gd-DTPA-SE and in the second sample ("M2"), there was used the distearylester Gd-DTPA-$(SE)_2$.

[0041] The mean size of the micelles and the micelle size distribution were determined by a Dynamic Light Scattering (DSL) method also known under the name of Photon Correlation Spectroscopy (PSC) using a Nicomp 370 HDL-NPSS apparatus. The particle size distribution (Gaussian) was measured (Nicomp) and found to have a peak at 150-170 nm SD ± 60-90 nm for both samples.

[0042] Two other samples were prepared from the same ingredients but the technique (mode 2) was modified as follows: the imaging species and the lipids were first dissolved in 25 ml of a 2/1 $CHCl_3$/MeOH mixture, the solution was evaporated to dryness as in Example 1, 20 ml of H_2O were added and dispersion was effected by sonication for 20 min, output 20. Then the F-108 was added and sonication resumed for 10 min. The sample with the monoester was labelled "M3", and that with the diester "M4". The particle size distribution was measured and found to have a peak at 70-80 nm SD ± 30-40 nm for both samples.

[0043] The r_1 and r_2 results are gathered in Table 2:

TABLE 2

	r_1	r2	size in nm
"M1	28.9	17.4	152 ± 63
"M2"	23.8	18.3	170 ± 90
"M3"	35.7	35.1	66 ± 36
"M4"	30.5	30.9	79 ± 38

[0044] It is speculated that the higher r_1 and r_2 values obtained with the mixed micelles according to "mode 2" may come from the fact that the micelles were smaller and had narrower size distribution than in the "mode 1".

Example 3

[0045] The experiments of Example 2 were repeated, using mode 2 and Gd-DTPA-SE but changing the nature of the phospholipid, i.e. using dipalmitoylphosphatidylglycerol (DPPG) and dipalmitoylphosphatidylcholine (DPPC). Table 3 gives the results obtained in terms of relaxivities r_1 and r_2 in $(mM.s)^{-1}$.

TABLE 3

Phospholipid	r_1	r_2	size in nm
DPPG-Na	30.2	28.6	110 ±50
DPPC	27.3	26.9	99 ±47
DPPA-Na	35.7	35.1	66 ±36

[0046] The experiments of Example 2 were repeated, using mode 2 and Gd-DTPA-$(SE)_2$ but changing the nature of the non-ionic surfactant, i.e. using

TABLE 4

Phospholipid	r_1	r_2	size in nm
DPPG-Na	29.4	28.8	77 ± 27
DPPC	21.6	21.5	36 ± 26
DPPA-Na	27.4	27.7	103 ± 31

eicosaethyleneglycol-octadecylether known under its trademark of BRIJ® 78 (Fluka). The results obtained in this experiment are given in Table 4.

Example 4

[0047] A composition was prepared using the directions of Example 2, mode (2) in 0.3M glycerol buffer (5 mM phosphate, pH 7.25). This contained per ml 5 mg of Gd-DTPA-SE, 10 mg of DPPA -Na and 10 mg of Synperonic® F-108.

[0048] First a calibration curve was constructed by diluting the composition with rat blood to a range of known Gd concentrations and measuring T_1 and T_2 for each concentration of Gd.

[0049] The composition was then injected intravenously into experimental rats (about 200 g) at the dose of 0.0385 mmol of Gd/kg (about 2 ml of suspension/animal). Two rats (making one group) were used in each experiment.

[0050] NMR relaxation measurements (T_1 and T_2) were carried out on 5 ml of the blood samples and the values (expressed in terms of Gd concentrations [Gd] by means of the calibration curve) were plotted against time to give the graph of Fig 4. The best mathematically fitting curve is given by the equation:

$$[Gd](mmol/l) = 0.5\ e^{-0.0157\ t\ (min)}$$

(showing a one compartment pharmacokinetic model).

[0051] The main pharmacokinetic parameters calculated from this one-compartment model were:

Elimination half-life = 44 min
Area under curve [AUC]0-∞ = 31.8 mM.min
Volume of distribution = 0.077 l/kg (or 77 ml/kg)
Clearance = 0.00121 l/kg.min

[0052] The elimination half-life (44 min) obtained for the micellar form is much longer i.e. better than that obtained for Gd-DTPA (15 min as $t^{1/2}$ (β)).

Example 5

[0053] An injectable composition was prepared according to Example 2, mode (2) using Gd-DTPA-$(SE)_2$ in place of Gd-DTPA-SE.

[0054] Then an *in-viva* experimental procedure was carried out in the rat as described in Example 4. The injected dose was 0.0345 mmol Gd/kg. The graph of Fig. 5 shows the results obtained.

[0055] The expression giving the [Gd] as a function of time was:

$$[Gd]\ (mmol/l) = 0.3\ e^{-0.0138\ t\ (min)}$$

[0056] The main pharmacokinetic parameters were:

Elimination half-life = 50 min
Area under curve [AUC]0-∞ = 21.7 mM.min
Volume of distribution = 0.115 l/kg (or 115 ml/kg)
Clearance = 0.00159 l/kg.min

[0057] There was virtually no difference between the results obtained with Gd-DTPA-SE and those obtained with Gd-DTPA-$(SE)_2$.

[0058] Much like in the Example 4, the elimination half-life (50 min) obtained for the micelles of the invention is much longer i.e. better than that obtained for Gd-DTPA (15 min as $t^{1/2}$ (β)).

Example 6

[0059] Injectable compositions were prepared according to Example 2, mode (2) using ^{153}Gd radioactive isotope. The following preparations were made:

^{153}Gd-DTPA-$(SE)_2$ /DPPA Na/Synperonic F-108
^{153}Gd-DTPA-$(SE)_2$ /DPPG Na/Synperonic F-108
^{153}Gd-DTPA-$(SE)_2$ /DPPC/Synperonic F-108

[0060] The ratio between the components 5:10:10 (mg/ml) was maintained the same for the three preparations.

[0061] The r_1 and r_2 values as well as the mean size distributions were close to the values obtained in Example 3 for the same compounds.

[0062] The preparations were injected into experimental rats (about 200 g) at the dose of 0.0234 mmol of Gd/kg (about 1 ml of suspension/animal) and blood samples taken 10,30,60,90 and 120 min after injection. The experiment was carried out on groups of 3 rats (one group per preparation). The radioactivity of the samples was measured using a γ-counter (Packard Minaxi γ). The change in concentration of Gd in mmol/l in the blood as a function of time for each preparation is shown in Fig. 6.

Example 7

[0063] Injectable ^{153} Gd-DTPA-$(SE)_2$/BRIJ® 78 and ^{153}Gd-DTPA-$(SE)_2$/DPPC/BRIJ® 78 compositions were prepared according to Example 2, mode (1) and mode (2) using ^{153}Gd radioactive isotope. The weight ratio of the components in the preparations was 5:10 and 5:10:10 respectively.

[0064] The preparations were injected into experimental rats at the dose of 0.0234 mmol of Gd/kg (about 1 ml of suspension/animal) and blood samples taken 10, 30, 60, 90 and 120 min after injection. The radioactivity of the samples was measured using a γ-counter (Packard Minaxi γ). From the plot of change in radioactivity of the samples shown in Fig. 7 it follows that when the preparations are made with BRIJ® 78, the presence of the phospholipid although beneficial in view of higher relaxivity and dwelling time, is not essential since the micelles of the surfactant and the paramagnetic complex are showing reasonably high relaxivity and stability in the circulation.

[0065] Relaxivities in $(mM.s)^{-1}$ obtained for the two preparations were:

TABLE 5

	ri	r2
DPPC/BRIJ® 78	21.6	21.5
BRIJ® 78	18.9	17.6

[0066] It was interesting to note that in the case of ^{153}Gd-DTPA-$(SE)_2$/BRIJ® 78 preparation the size of the micelles measured was about 538 ± 190 nm i.e. much greater than for the preparations in previous examples.
[0067] When the above experiments were repeated with Synperonic® F 108 in place of Brij®, it was found that the compositions obtained were more stable if the phospholipids were present.

Example 8

[0068] Injectable compositions were prepared according to Example 2, (mode 2) using the following lipophilic chelates:

Gd-DTPA-SA =	Gd-DTPA-Stearylamide
Gd-DTPA-(SA)2 =	Gd-DTPA-Distearylamide
Gd-DTPA-ME =	Gd-DTPA-Myristylester
Gd-DTPA-(ME)2 =	Gd-DTPA-Dimyristylester
Gd-DTPA-OE =	Gd-DTPA-Octylester
Gd-DTPA-(Ad)2 =	Gd-DTPA-Diadamantylamide
Gd-DOTA-SE =	Gd-DOTA-Stearylester
Gd-DOTA-PE =	Gd-DOTA-Palmitylester
Gd-HP-DO3A-SE =	Gd-HP-DO3A-Stearoylester

[0069] The r_1 and r_2 values as well as the mean size distribution measured were within the range of values obtained for Gd-DTPA-SE and Gd-DTPA-$(SE)_2$ compounds.

FIG. 2 Sources

[0070] For Relaxivities of Gd-DTPA, Dextran-(Gd-DTPA), Albumin-(Gd-DTPA) & Polylysine-(Gd-DTPA) see R.C. BRASH *Magnetic Resonance in Medicine 22* (1991) 282-287 and for relaxivity of MPEG-Polylyine-(Gd-DTPA) see A.A. Bogdanov et al. *Radiology* **187** (1993) 701-706.

Claims

1. An injectable NMR imaging composition comprising as components of a dispersion in a physiologically acceptable aqueous carrier phase a paramagnetic metal ion, a chelating agent having a lipophilic moiety, **characterised in that** the composition comprises a physiologically acceptable non-ionic surfactant or a mixture of non-ionic surfactants wherein the non-ionic surfactant is a block-copolymer having polyoxyethylene and polyoxypropylene segments, a polyethyleneglycolalkylether, a polyoxyethylene fatty acid ester, an n-alkylglucopyranoside, or an n-alkyl maltotrioside and optionally one or more amphipatic compounds, the components of the dispersion being in a micellar form.

2. The composition of claim 1, wherein the micelles are mixed micelles with the particle size between 10 and 800 nm.

3. The composition of claim 2, wherein the micelles are mixed micelles with the particle size preferably between 30 and 500 nm.

4. The composition of claim 1, wherein the surfactant is Pluronic®, Poloxamer®, Poloxamine®, Synperonic®, BRIJ®, Myrj® or mixtures thereof.

5. The composition of claim 1, wherein the surfactant is BRIJ®.

6. The composition of claim 1, wherein the amphipatic compound is a dialkyl glycerophospholipid in which alkyl has at least twelve carbon atoms.

7. The composition of claim 6, wherein the phospholipid is selected from phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, cardiolipin and sphingomyelin.

8. The composition of claim 7, wherein the phospholipid consists of a monophosphate ester of a substituted or partially substituted glycerol, at least one functional group of said glycerol being esterified by saturated or unsaturated aliphatic fatty acid, or etherified by saturated or unsaturated alcohol, the other two acidic functions of the phosphoric acid being either free or salified with alkali or earth-alkali metals.

9. The composition of claim 8, wherein the phospholipid is a monophosphate of a fatty acid glyceride selected from dimyristoyl-phosphatidic acid, dipalmitoylphosphatidic acid, or distearoylphosphatidic acid.

10. The composition of claim 1, wherein the amphipatic compound comprises two or more compounds selected from ionic and neutral phospholipids, mono alkyl or alkenyl esters of phosphoric acid and/or cholesterol, ergosterol, phytosterol, sitosterol, lanosterol, and tocopherol.

11. The composition of any one of preceding claims, wherein the lipophilic moiety of the imaging agent is a C_1 to C_{24} alkyl or alkylene group or substituted

or unsubstituted benzyl- or phenyl alkyl group.

12. The composition of claim 11, wherein the lipophilic moiety of the imaging agent is a carboxylate ester of saturated and unsaturated C_1 to C_{24} aliphatic or aromatic alcohols or is a carboxylate amide of saturated and unsaturated C_1 to C_{24} aliphatic or aromatic amines.

13. The composition of claim 12, wherein the alcohol is methanol, ethanol, propanol, butanol (n-, iso-, tert-), pentanol, hexanol and isomers, heptanol, octanol, nonanol, decanol and isomers, fatty alcohols, substituted and unsubstituted benzyl- and higher phenylalkyl-alcohols.

14. The composition of claim 12, wherein the amine is methylamine, ethylamine, propylamine, butylamine (n-, iso-, tert-), pentylamine, hexylamine (and isomers), heptylamine, octylamine (and isomers), nonylamine, decylamine, aminoadamantane, fatty amines and substituted and unsubstituted benzyl- and higher phenylalkyl-amines.

15. The composition of claim 11, wherein the lipophilic moiety of the imaging agent is provided with lipophilic hydrophobic groups linked to the alkylene segments of the molecular back-bone, to the α-carbon of the carboxylate functions or to a hydroxyl group when present in the chelating agent.

16. The composition of claim 1, wherein the chelating agent is selected from EDTA, DTPA, BOPTA, DOTA, DO3A and/or derivatives thereof.

17. The composition of claim 1, wherein the paramagnetic metal ion is selected from Gd(III), Mn(II), Cr (III), Cu(II), Fe (III), Pr(III), Nd(III) Sm(III), Tb(III), Yt (III) Dy(III), Ho(III) and Er(III).

18. The composition of claims 1-17, wherein the weight ratio of the lipophilic imaging agent and the surfactant in the composition is between 1:10 and 10: 1, preferably between 1:3 and 3:1.

19. The composition of claim 18, wherein the weight ratio of the lipophilic imaging agent and the surfactant in the composition is preferably between 1:3 and 3: 1.

20. The composition of claim 7-17, wherein the weight ratio of the phospholipid to the surfactant is from 1: 10 to 10:1 and preferably between 1:2 and 2:1.

21. A dry, pulverulent formulation comprising as components a paramagnetic metal ion, a chelating agent having a lipophilic moiety, a physiologically acceptable non-ionic surfactant or a mixture of non-ionic surfactants and optionally one or more amphipatic compounds, which upon dispersion in a physiologically acceptable liquid carrier, will form a micellar dispersion useful as a blood pool NMR imaging contrast agent.

22. An injectable aqueous suspension comprising the composition of claims 1-20 suspended in a physiologically acceptable liquid carrier useful as a NMR blood pool contrast agent.

23. A method for making composition of claims 1-20, **characterised by** the steps of:

a) selecting and suspending a complex of a paramagnetic metal ion, a chelating agent comprising a lipophilic moiety, one or more non-ionic surfactants, and optionally one or more amphipatic compounds and in an aqueous phase to form a mixture, and

b) energising the mixture by sonicating or microfluidizing to bring the ingredients into intimate contact and produce homogeneous dispersion of the components in micellar form.

24. The method of claim 23, wherein after the sonication or microfluidization the mixture is sterilised and/ or lyophilised.

25. The method of claim 23 or 24, wherein the surfactant is added to the mixture of the compound after said energising and optionally repeating the sonication or microfluidization.

26. The method of claim 23, wherein the amphipatic compound is a phospholipid consisting of monophosphate of a fatty acid glyceride selected from dimyristoylphosphatidic acid, dipalmitoylphosphatidic acid, or distearoylphosphatidic acid.

27. The composition of claims 1-20, for use in NMR blood pool imaging of organs in human or animal body.

28. Use of the pulverulent formulation of claim 21 for the manufacture of an MRI contrast agent.

29. A two component kit comprising, as the first component, a dry formulation of claim 21 stored under an inert atmosphere and, as the second component, a physiologically acceptable carrier liquid which when admixed with the first component provides, as a suspension of the two components, an injectable NMR contrast composition of claims 1-20.

Patentansprüche

1. Injizierbare NMR-Bilderzeugungszusammensetzung, die als Bestandteile einer Dispersion in einer physiologisch verträglichen wässrigen Trägerphase ein paramagnetisches Metallion, einen Chelatbildner mit einer lipophilen Gruppierung enthält, **dadurch gekennzeichnet, dass** die Zusammensetzung ein physiologisch verträgliches nicht-ionisches grenzflächenaktives Mittel oder eine Mischung von nicht-ionischen grenzflächenaktiven Mitteln, wobei das nicht-ionische grenzflächenaktive Mittel ein BlockCopolymer mit Polyoxyethylen- und Polyoxypropylen-Abschnitten, ein Polyethylenglykolalkylether, ein Polyoxyethylenfettsäureester, ein n-Alkylglucopyranosid oder ein n-Alkylmaltotriosid ist, und gegebenenfalls eine oder mehrere amphipathische Verbindungen enthält, wobei die Bestandteile der Dispersion in einer Mizellenform vorliegen.

2. Zusammensetzung nach Anspruch 1, wobei die Mizellen gemischte Mizellen mit einer Teilchengröße zwischen 10 und 800 nm sind.

3. Zusammensetzung nach Anspruch 2, wobei die Mizellen gemischte Mizellen mit einer Teilchengröße vorzugsweise zwischen 30 und 500 nm sind.

4. Zusammensetzung nach Anspruch 1, wobei das grenzflächenaktive Mittel Pluronic®, Poloxamer®, Poloxamine®, Synperonic®, BRIJ®, Myrj® oder eine Mischung davon ist.

5. Zusammensetzung nach Anspruch 1, wobei das grenzflächenaktive Mittel BRIJ® ist.

6. Zusammensetzung nach Anspruch 1, wobei die amphipathische Verbindung ein Dialkylglycerophospholipid ist, in welchem Alkyl mindestens zwölf Kohlenstoffatome aufweist.

7. Zusammensetzung nach Anspruch 6, wobei das Phospholipid aus Phosphatidsäure, Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylserin, Phosphatidylglycerin, Phosphatidylinositol, Cardiolipin und Sphingomyelin ausgewählt ist.

8. Zusammensetzung nach Anspruch 7, wobei das Phospholipid aus einem Monophosphatester eines substituierten oder partiell substituierten Glycerins besteht, wobei mindestens eine funktionelle Gruppe des Glycerins durch gesättigte oder ungesättigte aliphatische Fettsäure verestert ist oder durch gesättigten oder ungesättigten Alkohol ver-ethert ist, wobei die anderen zwei sauren Funktionen der Phosphorsäure entweder frei sind oder ein Salz mit Alkali- oder Erdalkalimetallen bilden.

9. Zusammensetzung nach Anspruch 8, wobei das Phospholipid ein Monophosphat eines Fettsäureglycerids ist, ausgewählt aus Dimyristoylphosphatidsäure, Dipalmitoylphosphatidsäure oder Distearoylphosphatidsäure.

10. Zusammensetzung nach Anspruch 1, wobei die amphipathische Verbindung zwei oder mehr Verbindungen enthält, ausgewählt aus ionischen und neutralen Phospholipiden, Monoalkyl- oder -alkenylestern von Phosphorsäure und/oder Cholesterin, Ergosterin, Phytosterin, Sitosterin, Lanosterin und Tocopherol.

11. Zusammensetzung nach einem der vorangegangenen Ansprüche, wobei die lipophile Gruppierung des bilderzeugenden Mittels eine C_1- bis C_{24}-Alkyl- oder -Alkylengruppe oder eine substituierte oder unsubstituierte Benzyl- oder Phenylalkylgruppe ist.

12. Zusammensetzung nach Anspruch 11, wobei die lipophile Gruppierung des bilderzeugenden Mittels ein Carboxylatester von gesättigten und ungesättigten aliphatischen C_1bis C_{24}-Alkoholen oder aromatischen Alkoholen ist oder ein Carboxylatamid von gesättigten und ungesättigten aliphatischen C_1- bis C_{24}-Aminen oder aromatischen Aminen ist.

13. Zusammensetzung nach Anspruch 12, wobei der Alkohol Methanol, Ethanol, Propanol, Butanol (n-, iso-, tert-), Pentanol, Hexanol und Isomere davon, Heptanol, Octanol, Nonanol, Decanol und Isomere davon, Fettalkohole, substituierte und unsubstituierte Benzyl- und höhere Phenylalkylalkohole ist.

14. Zusammensetzung nach Anspruch 12, wobei das Amin Methylamin, Ethylamin, Propylamin, Butylamin (n-, iso-, tert-), Pentylamin, Hexylamin (und Isomere davon), Heptylamin, Octylamin (und Isomere davon), Nonylamin, Decylamin, Aminoadamantan, Fettamine und substituierte und unsubstituierte Benzyl- und höhere Phenylalkylamine ist.

15. Zusammensetzung nach Anspruch 11, wobei die lipophile Gruppierung des bilderzeugenden Mittels mit lipophilen hydrophoben Gruppen versehen ist, die an die Alkylenabschnitte der Molekülhauptkette, an das α-Kohlenstoffatom der Carboxylatfunktionen oder an eine Hydroxylgruppe, sofern eine in dem Chelatbildner vorhanden ist, gebunden sind.

16. Zusammensetzung nach Anspruch 1, wobei der Chelatbildner aus EDTA, DTPA, BOPTA, DOTA, DO3A und/oder Derivaten davon ausgewählt ist.

17. Zusammensetzung nach Anspruch 1, wobei das paramagnetische Metallion aus Gd(III), Mn(II), Cr (III), Cu(II), Fe(III), Pr(III), Nd(III), Sm(III), Tb(III), Yt

(III), Dy(III), Ho(III) und Er(III) ausgewählt ist.

18. Zusammensetzung nach den Ansprüchen 1-17, wobei das Gewichtsverhältnis des lipophilen bilderzeugenden Mittels und des grenzflächenaktiven Mittels in der Zusammensetzung zwischen 1:10 und 10:1, vorzugsweise zwischen 1:3 und 3:1 liegt.

19. Zusammensetzung nach Anspruch 18, wobei das Gewichtsverhältnis des lipophilen bilderzeugenden Mittels und des grenzflächenaktiven Mittels in der Zusammensetzung vorzugsweise zwischen 1:3 und 3:1 liegt.

20. Zusammensetzung nach Anspruch 7-17, wobei das Gewichtsverhältnis des Phospholipids zu dem grenzflächenaktiven Mittel 1:10 bis 10:1, vorzugsweise zwischen 1:2 und 2:1 beträgt.

21. Trockene, pulverförmige Formulierung, die als Bestandteile ein paramagnetisches Metallion, einen Chelatbildner mit einer lipophilen Gruppierung, ein physiologisch verträgliches nicht-ionisches grenzflächenaktives Mittel oder eine Mischung von nicht-ionischen grenzflächenaktiven Mitteln und gegebenenfalls eine oder mehrere amphipathische Verbindungen enthält, die beim Dispergieren in einem physiologisch verträglichen flüssigen Träger eine Mizellendispersion bilden, die als ein NMR-Bilderzeugungskontrastmittel für den Blutpool nützlich ist.

22. Injizierbare wässrige Suspension, enthaltend die in einem physiologisch verträglichen flüssigen Träger suspendierte Zusammensetzung nach den Ansprüchen 1-20, die als NMR-Blutpoolkontrastmittel nützlich ist.

23. Verfahren zur Herstellung einer Zusammensetzung nach den Ansprüchen 1-20, **gekennzeichnet durch** die Schritte:

    a) Auswählen und Suspendieren eines Komplexes eines paramagnetischen Metallions, eines eine lipophile Gruppierung enthaltenden Chelatbildners, eines nicht-ionischen grenzflächenaktiven Mittels bzw. mehrerer nicht-ionischer grenzflächenaktiver Mittel und gegebenenfalls einer oder mehrerer amphipathischer Verbindungen und in einer wässrigen Phase, um eine Mischung zu bilden, und

    b) Aktivieren der Mischung **durch** Beschallen oder Mikrofluidisieren, um die Bestandteile in engen Kontakt zu bringen und eine homogene Dispersion der Bestandteile in Mizellenform zu erzeugen.

24. Verfahren nach Anspruch 23, wobei nach dem Beschallen oder Mikrofluidisieren die Mischung sterilisiert und/oder lyophilisiert wird.

25. Verfahren nach Anspruch 23 oder 24, wobei das grenzflächenaktive Mittel der Mischung der Verbindung nach dem Aktivieren zugesetzt und das Beschallen oder Mikrofluidisieren gegebenenfalls wiederholt wird.

26. Verfahren nach Anspruch 23, wobei die amphipathische Verbindung ein Phospholipid bestehend aus dem Monophosphat eines Fettsäureglycerids ist, ausgewählt aus Dimyristoylphosphatidsäure, Dipalmitoylphosphatidsäure oder Distearoylphosphatidsäure.

27. Zusammensetzung nach den Ansprüchen 1-20 für eine Verwendung bei der NMR-Bilderzeugung vom Blutpool von Organen im menschlichen oder tierischen Körper.

28. Verwendung der pulverförmigen Formulierung nach Anspruch 21 für die Herstellung eines MRI-Kontrastmittels.

29. Zwei-Komponenten-Kit, der als erste Komponente eine trockene Formulierung nach Anspruch 21, die unter einer inerten Atmosphäre gelagert wird, und als zweite Komponente eine physiologisch verträgliche Trägerflüssigkeit enthält, die, wenn sie mit der ersten Komponente vermischt wird, als eine Suspension der zwei Komponenten eine injizierbare NMR-Kontrastzusammensetzung nach den Ansprüchen 1-20 ergibt.

Revendications

1. Composition d'imagerie RMN injectable comprenant comme composants d'une dispersion dans une phase support aqueuse physiologiquement acceptable un ion métallique paramagnétique, un agent chélateur ayant une partie lipophile, **caractérisée en ce que** la composition comprend un tensioactif non ionique physiologiquement acceptable ou un mélange de tensioactifs non ioniques, le tensioactif non ionique étant un copolymère bloc ayant des segments de polyoxyéthylène et de polyoxypropylène, un alkyléther de polyéthylène glycol, un ester d'acide gras de polyoxyéthylène, un n-alkylglucopyranoside ou un n-alkylmaltotrioside et éventuellement un ou plusieurs composés amphipatiques, les composants de la dispersion étant sous une forme micellaire.

2. Composition selon la revendication 1, dans laquelle les micelles sont des micelles mélangées avec une taille de particules située entre 10 et 800 nm.

3. Composition selon la revendication 2, dans laquelle les micelles sont des micelles mélangées avec une taille de particules située entre 30 et 500 nm.

4. Composition selon la revendication 1, dans laquelle le tensioactif est le Pluronic®, le Poloxamer®, la Poloxamine®, le Synperonic®, BRIJ®, Myrj® ou des mélanges de ceux-ci.

5. Composition selon la revendication 1, dans laquelle le tensioactif est le BRIJ®.

6. Composition selon la revendication 1, dans laquelle le composé amphipatique est un glycérophospholipide dont l'alkyle possède au moins douze atomes de carbone.

7. Composition selon la revendication 6, dans laquelle le phospholipide est choisi parmi l'acide phosphatidique, la phosphatidylcholine, la phosphatidyléthanolamine, la phosphatidylsérine, le phosphatidylglycérol, le phosphatidylinositol, la cardiolipine et la sphingomyéline.

8. Composition selon la revendication 7, dans laquelle le phospholipide est constitué d'un ester de monophosphate d'un glycérol substitué ou partiellement substitué, au moins un groupe fonctionnel dudit glycérol étant estérifié par un acide gras aliphatique saturé ou insaturé ou étherifié par un alcool saturé ou insaturé, les deux autres fonctions acides de l'acide phosphorique étant soit libres soit dotées de sels de métaux alcalins ou alcalino-terreux.

9. Composition selon la revendication 8, dans laquelle le phospholipide est un monophosphate d'un glycéride d'acide gras sélectionné parmi l'acide dimyristoylphosphatidique, l'acide dipalmitoylphosphatidique ou l'acide distéaroylphosphatidique.

10. Composition selon la revendication 1, dans laquelle le composé amphipatique comprend deux composés ou plus choisis parmi les phospholipides ioniques et neutres, les monoesters alkyles ou alcènyles d'acide phosphorique et/ou le cholestérol, l'ergostérol, le phytostérol, le sitostérol, le lanostérol et le tocophérol.

11. Composition selon l'une quelconque des revendications précédentes, dans laquelle la partie lipophile de l'agent d'imagerie est un groupe alkyle en C_1 à C_{24} ou alkylène ou un groupe alkyle de benzyle ou phényle substitué ou non substitué.

12. Composition selon la revendication 11, dans laquelle la partie lipophile de l'agent d'imagerie est un ester de carboxylate d'alcools aromatiques ou aliphatiques en C_1 à C_{24} saturés et insaturés ou est un amide de carboxylate d'amines aromatiques ou aliphatiques en C_1 à C_{24} saturées et insaturées.

13. Composition selon la revendication 12, dans laquelle l'alcool est le méthanol, l'éthanol, le propanol, le butanol (n-, iso-, tert-), le pentanol, l'hexanol et leurs isomères, l'heptanol, l'octanol, le nonanol, le décanol et leurs isomères, les alcools gras, les alcools de benzyle et alcools supérieurs de phénylalkyle substitués ou non substitués.

14. Composition selon la revendication 12, dans laquelle l'amine est la méthylamine, l'éthylamine, la propylamine, la butylamine (n-, iso-, tert-), la pentylamine, l'hexylamine (et leurs isomères), l'héptylamine, l'octylamine (et leurs isomères), la nonylamine, la décylamine, l'aminoadamantane, les amines grasses et les amines de benzyle et amines supérieures de phénylalkyle substituées et non substituées.

15. Composition selon la revendication 11, dans laquelle la partie lipophile de l'agent d'imagerie est munie de groupes hydrophobes lipophiles liés aux segments alkylènes du squelette moléculaire, au carbone α des fonctions carboxylates ou à un groupe hydroxyle quand il est présent dans l'agent chélateur.

16. Composition selon la revendication 1, dans laquelle l'agent chélateur est choisi parmi EDTA, DTPA, BOPTA, DOTA, DO3A et/ou dérivés de ceux-ci.

17. Composition selon la revendication 1, dans laquelle l'ion métallique paramagnétique est choisi parmi Gd (III), Mn(II), Cr(III), Cu(II), Fe(III), Pr(III), Nd(III), Sm (III), Tb(III), Yt(III), Dy(III) et Er(III).

18. Composition selon les revendications 1 à 17, dans laquelle le rapport massique de l'agent d'imagerie lipophile et du tensioactif dans la composition se situe entre 1 : 10 et 10 : 1, de préférence entre 1 : 3 et 3 : 1.

19. Composition selon la revendication 18, dans laquelle le rapport massique de l'agent d'imagerie lipophile et du tensioactif dans la composition est situé de préférence entre 1 :3 et 3 :1.

20. Composition selon les revendication 7 à 17, dans laquelle le rapport massique du phospholipide sur le tensioactif dans la composition est situé entre 1 : 10 et 10 :1, de préférence entre 1 : 2 et 2 : 1.

21. Formulation pulvérulente sèche comprenant comme composants un ion métallique paramagnétique, un agent chélateur ayant une partie lipophile, un tensioactif non ionique physiologiquement accepta-

ble ou un mélange de tensioactifs non ioniques et éventuellement un ou plusieurs composés amphipatiques, qui, lors de la dispersion dans un support liquide physiologiquement acceptable, vont former une dispersion micellaire utile comme agent de contraste d'imagerie RMN de pool sanguin.

22. Suspension aqueuse injectable comprenant la composition selon les revendications 1 à 20 suspendue dans un support liquide physiologiquement acceptable, utile comme agent de contraste RMN de pool sanguin.

23. Procédé de préparation de la composition selon les revendications 1 à 20, **caractérisé par** les étapes consistant à :

a) sélectionner et suspendre un complexe d'un ion métallique paramagnétique, un agent chélateur comprenant une partie lipophile, un ou plusieurs tensioactifs non ioniques et éventuellement un ou plusieurs composés amphipatiques et en phase aqueuse pour former un mélange, et
b) énergiser le mélange par sonication ou par microfluidisation pour amener les ingrédients en entrer en contact intime et produire une dispersion homogène des composants sous forme micellaire.

24. Procédé selon la revendication 23, dans lequel, après sonication ou la microfluidisation, le mélange est stérilisé et/ou lyophilisé.

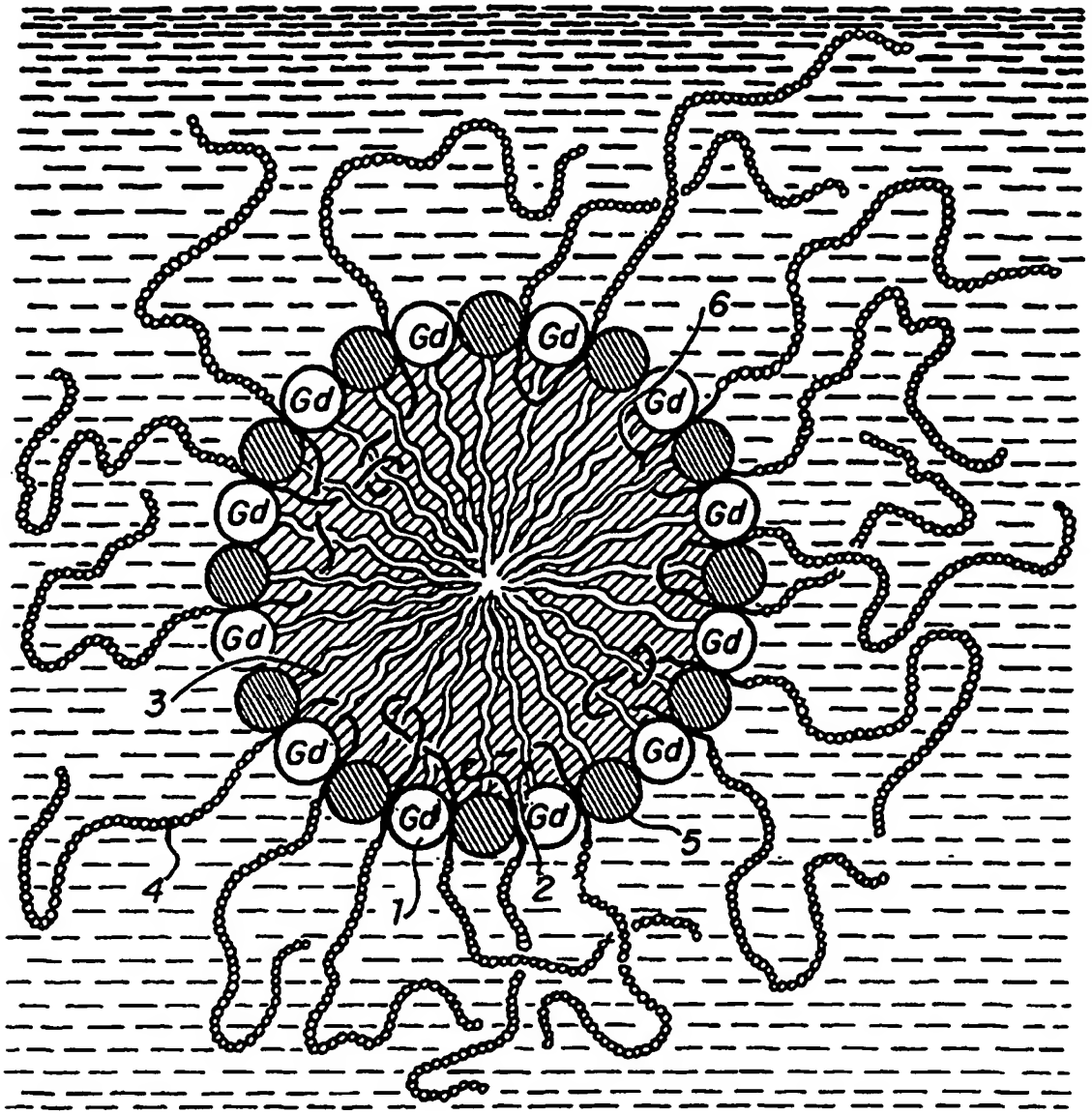
25. Procédé selon la revendication 23 ou 24, dans lequel le tensioactif est ajouté au mélange du composé après ladite énergisation et éventuellement la répétition de la sonication ou de la microfluidisation.

26. Procédé selon la revendication 23, dans lequel le composé amphipatique est un phospholipide constitué d'un monophosphate d'un glycéride d'acide gras choisi parmi l'acide dimyristoylphosphatidique, l'acide dipalmitoylphosphatidique ou l'acide distéaroylphosphatidique.

27. Composition selon les revendications 1 à 20, destinée à être utilisée dans l'imagerie RMN de pool sanguin des organes d'un être humain ou animal.

28. Utilisation de la formulation pulvérulente selon la revendication 21 pour la fabrication d'un agent de contraste IRM.

29. Kit à deux composants comprenant, comme premier composant, une formulation sèche selon la revendication 21 stockée sous atmosphère inerte et, comme deuxième composant, un liquide support physiologiquement acceptable qui, une fois ajouté au premier composant, donne une composition de contraste RMN injectable selon les revendications 1 à 20, sous la forme d'une suspension de deux composants.



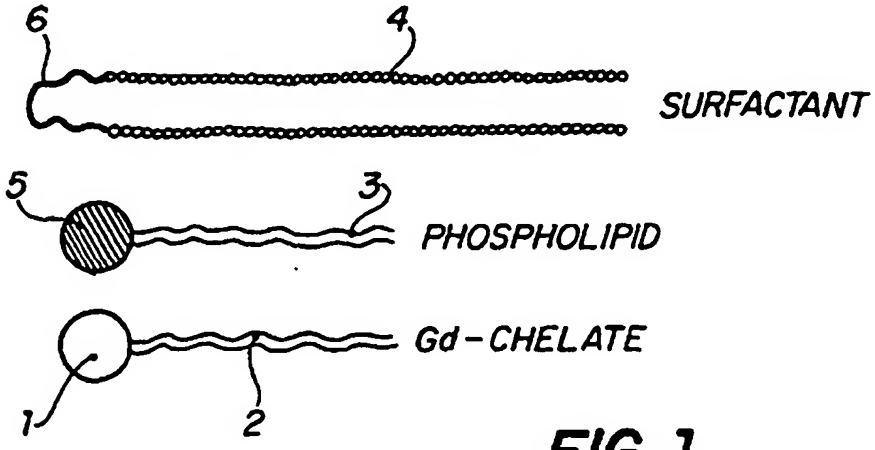


FIG. 1

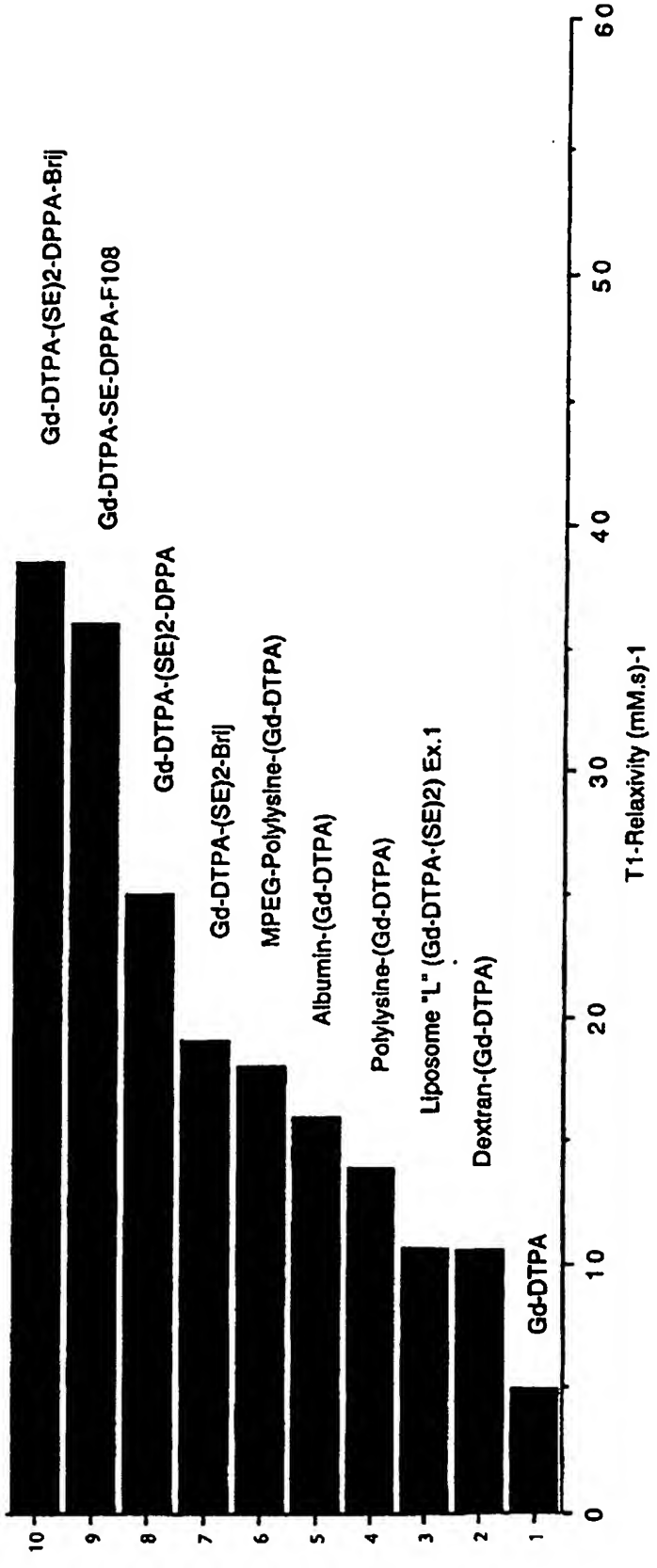

Gd-DTPA-(SE)2-DPPA-Brij
Gd-DTPA-SE-DPPA-F108
Gd-DTPA-(SE)2-DPPA
Gd-DTPA-(SE)2-Brij
MPEG-Polylysine-(Gd-DTPA)
Albumin-(Gd-DTPA)
Polylysine-(Gd-DTPA)
Liposome "L" (Gd-DTPA-(SE)2) Ex.1
Dextran-(Gd-DTPA)
Gd-DTPA
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
T1-Relaxivity (mM.s)-1

Fig. 2

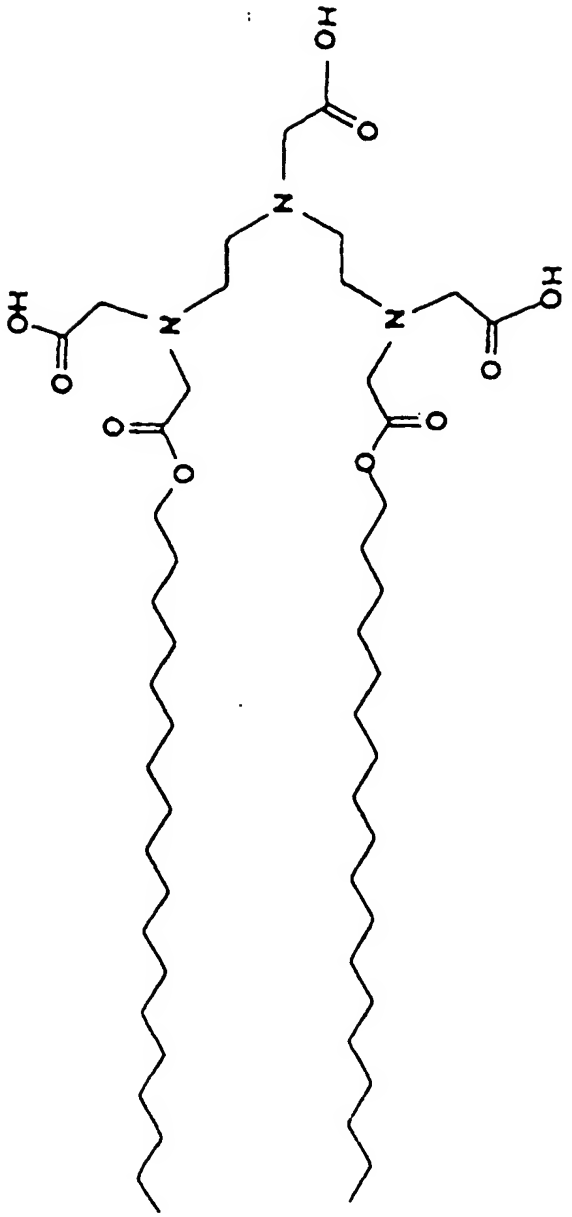


FIG.3

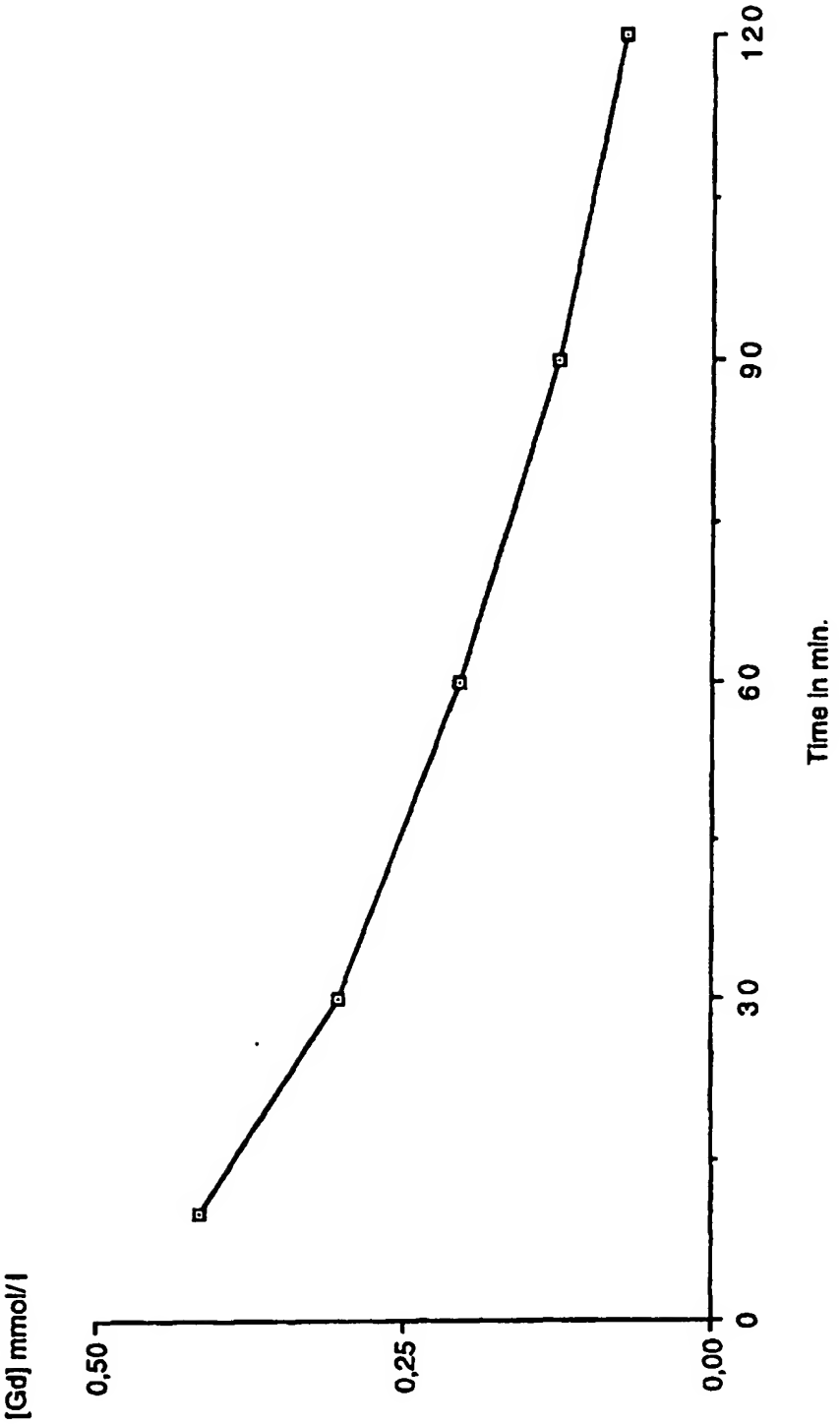


FIG. 4

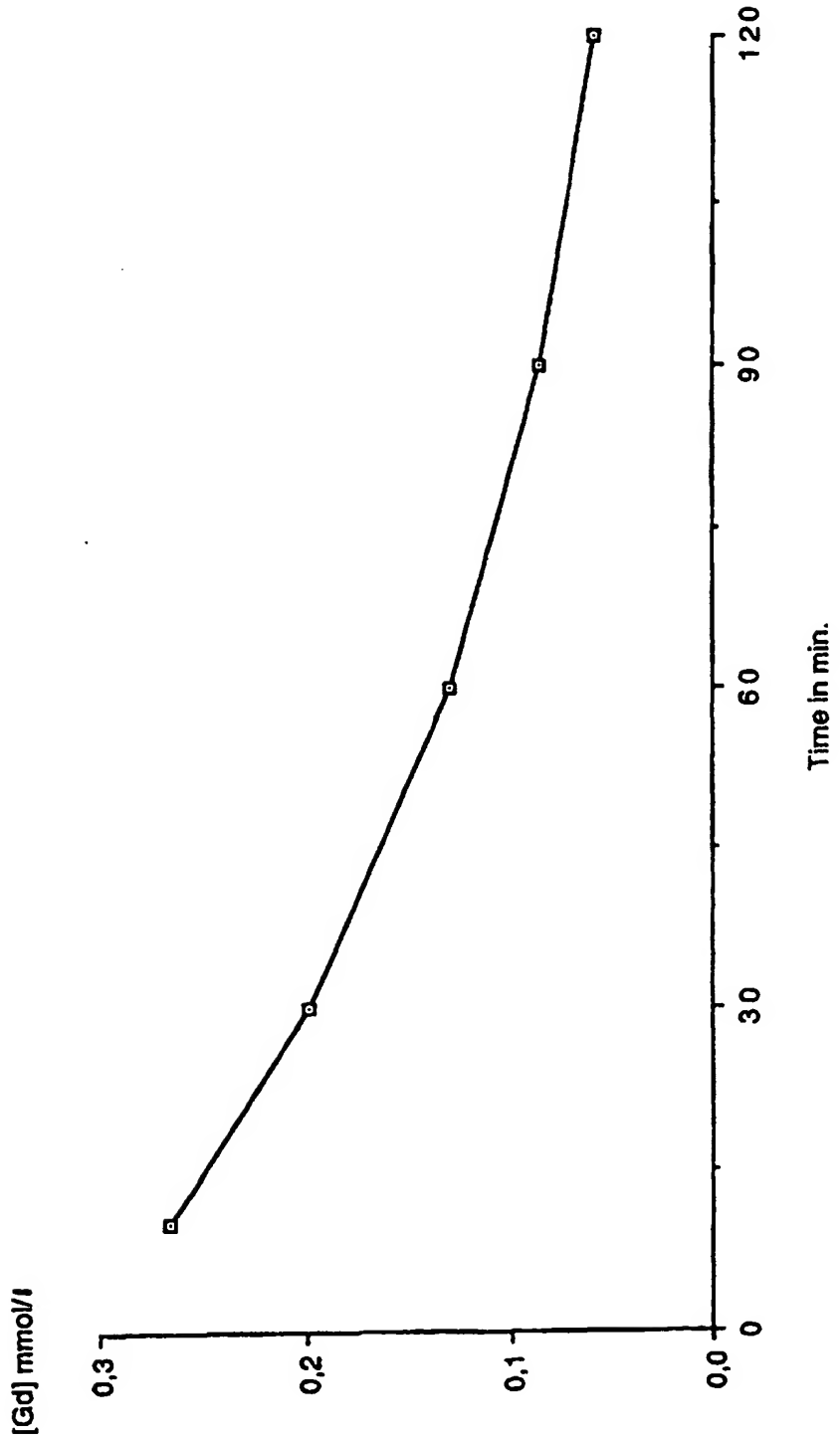


FIG. 5

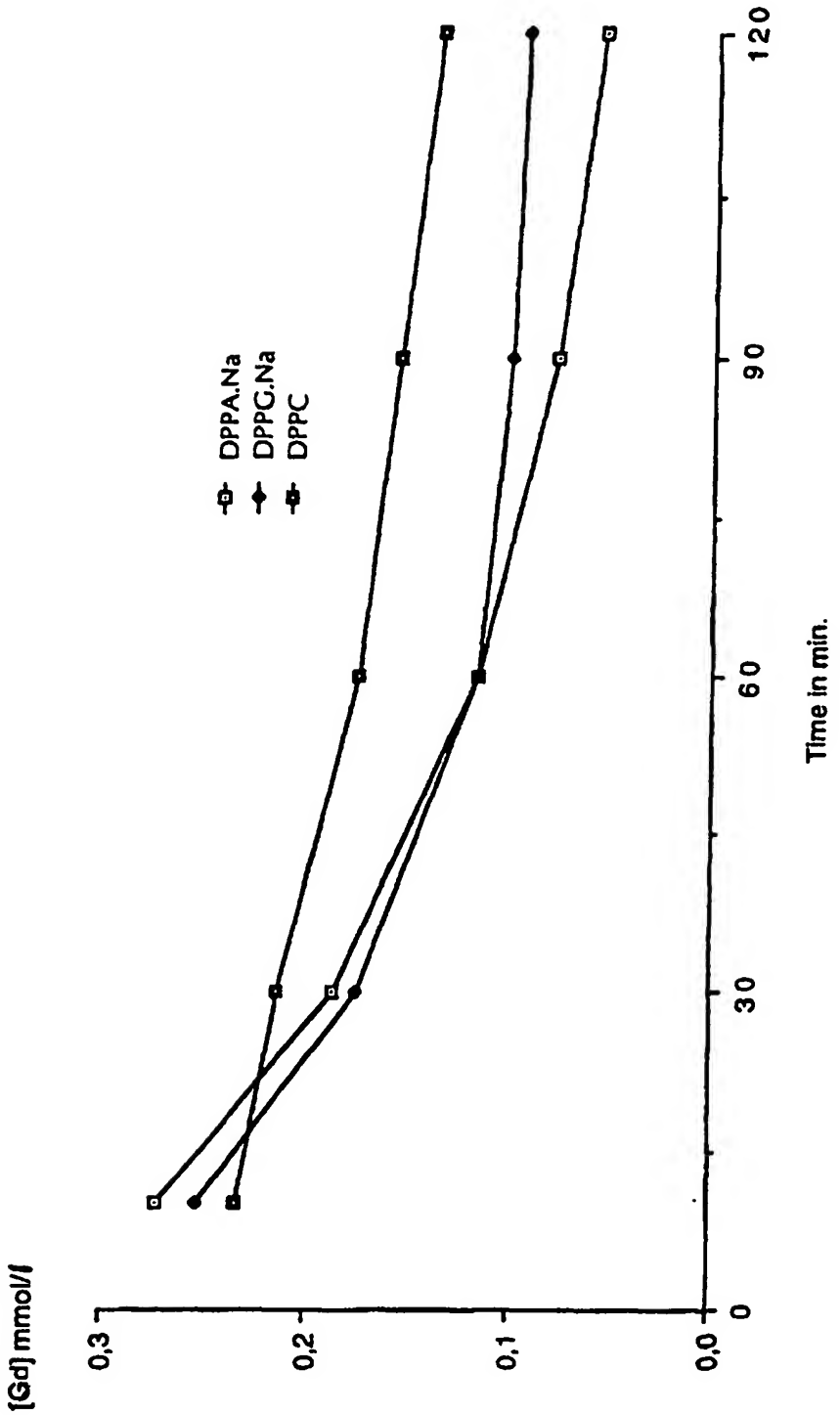

DPPA.Na
DPPG.Na
DPPC
[Gd] mmol/l
0,3
0,2
0,1
0,0
0
30
60
90
120
Time in min.

FIG. 6

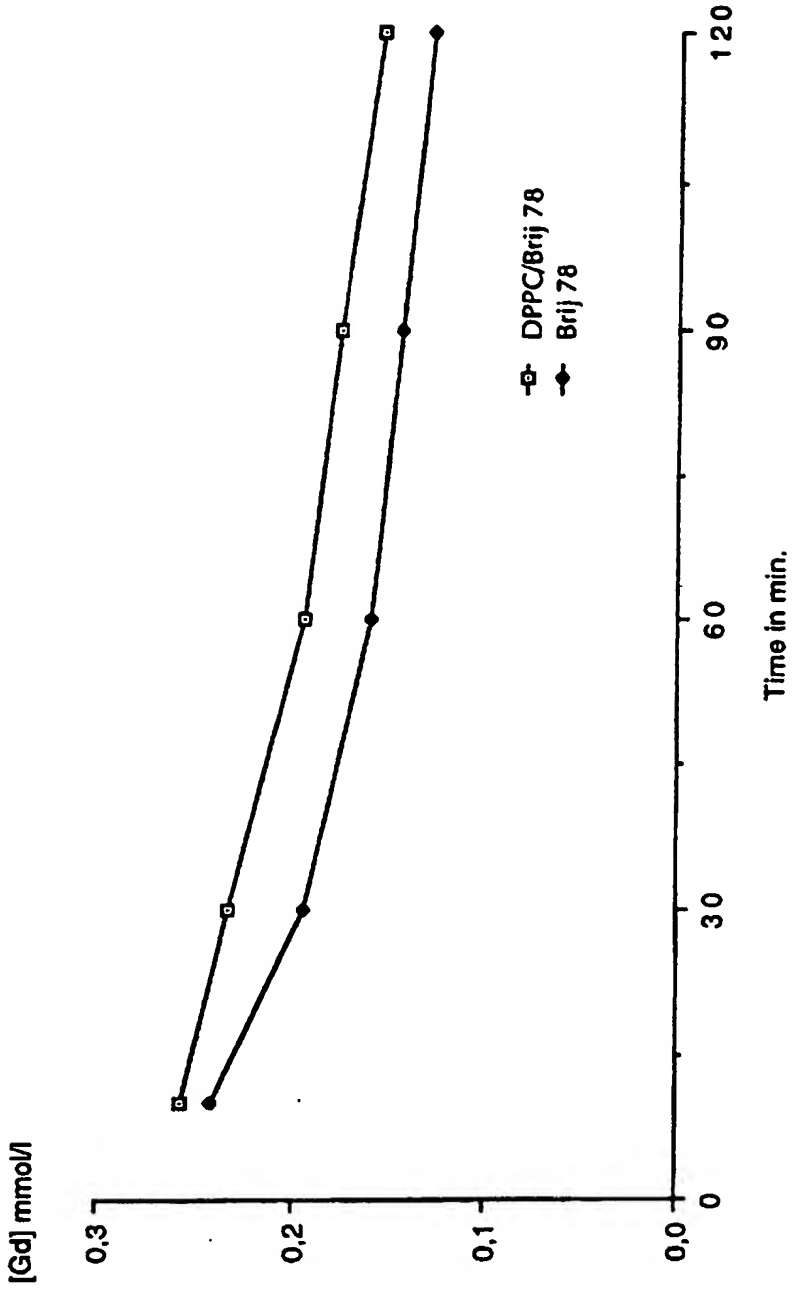

[Gd] mmol/l
0,3
0,2
0,1
0,0
0
30
60
90
120
Time in min.
DPPC/Brij 78
Brij 78

FIG. 7